

Allonais inaequalis (Annelida: Oligochaeta: Tubificidae) in North America

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Abstract.—The oligochaete *Allonais inaequalis* (Stephenson, 1911), previously thought to be unknown in North America, was found in the effluent of a sewage treatment plant in New Jersey and recently in the Greater Miami River in Ohio. It is redescribed from this material and compared to the other two species, *A. paraguayensis* (Michaelsen, 1905) and *A. pectinata* (Stephenson, 1910) reported from North America. Identifications of specimens collected from North America, previously thought to be *A. paraguayensis*, were determined to be incorrect; thus, this species is not yet known to occur in North America. *Nais magnaseta* Harman, 1973, which closely resembles *A. pectinata*, also is discussed.

The naidid oligochaete¹ *Allonais inaequalis* (Stephenson, 1911) was first described as *Nais pectinata* var. *inaequalis* Stephenson, 1911. Sperber (1948) erected the genus *Allonais*, using *A. inaequalis* as the generic type. She distinguished *Allonais* from *Nais* by the absence of eyes, by the anterior and posterior ventral chaetae being of similar size and shape, by no discernable stomachal dilatation, by a vascular plexus in the anterior segments, by no prostate gland cells, and by no formation of budding zones. She also indicated that *Allonais*, unlike *Nais*, was confined to the tropics. She

included five species (all formerly found in *Nais*) in this new genus, including two, *A. pectinata* (Stephenson, 1910) and *A. paraguayensis*, (Michaelsen, 1905), subsequently reported from North America (Brinkhurst & Jamieson 1971, Brinkhurst 1986). Brinkhurst (1986) included *A. inaequalis* in his North American key, suggesting that it probably occurred in North America due to its worldwide distribution and because drawings of the chaetae of *A. paraguayensis* closely resembled the chaetae of *A. inaequalis* (e.g., Hiltunen & Klemm 1980). New material collected and identified by the authors, or sent to the authors for identification or verification, encouraged us to reassess the status of this genus in North America.

Materials and Methods

Oligochaetes were observed swimming in the effluent of the Linden-Roselle Sewage Authority wastewater treatment plant in New Jersey, U.S.A., in October 1999. The facility is a typical tertiary treatment plant,

¹ Based on sequences of 18S rDNA and other molecular and morphological data, Erséus et al. (2002) concluded that the family Naididae is polyphyletic and that the species of naidids are more correctly placed within a subfamily of the Tubificidae. Therefore, Naididae has become a junior synonym of the family Tubificidae. As the name Naididae is older than Tubificidae, the authors are requesting that Tubificidae take precedence because of the large number of species in the family compared to those within the naidids and await a ruling by the International Commission on Zoological Nomenclature (C. Erséus, pers. comm.; Erséus & Gustavsson 2002).

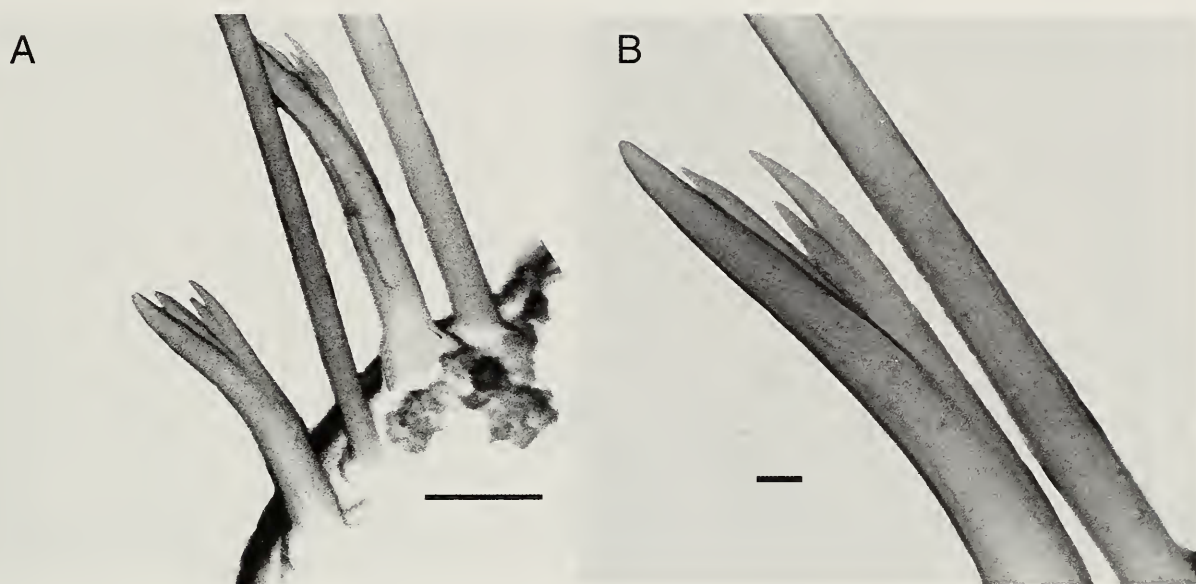


Fig. 1. *Allonais inaequalis* (SEM). A, dorsal chaetal bundle; B, same as A, enlarged. Scale bars: A = 5 μm . B = 1 μm .

treating approximately 80% residential and 20% municipal waste. Specimens were collected using a small bucket in the uptake valve in the aeration ponds immediately prior to chlorination. Half of the worms were fixed in 10% formalin; the remainder were fixed in 70% ethanol. One oligochaete specimen collected in July 2001 from the Greater Miami River, Ohio, U.S.A., tentatively identified as *A. paraguayensis*, was sent to the senior author for verification.

The description of *A. inaequalis* below is based on 10 specimens from the New Jersey material, preserved in ethanol, and mounted on slides using CMCP mounting medium for observations using light microscopy. Measurements were made using a calibrated ocular scale on a Leitz Laborlux-12 compound microscope, and drawings were made using a camera lucida drawing tube with the same microscope. Oligochaetes fixed in formalin from New Jersey material were prepared for scanning electron microscopy (SEM). Specimens were mounted on aluminum stubs by placing one to two specimens in a drop of water on each stub that had been prepared with transparent double-coated sticky tape (No. 666, 3M Corp.). Stubs were immersed in liquid nitrogen to instantly freeze the specimens, then placed in an Edwards-Pearse

Model EPD3 tissue dryer (temperature -64°C ; vacuum 0.0002 torr) for subsequent sublimation of liquid associated with the specimens. After sublimation was complete (4–6 hr), stubs were placed in an SPI Module Sputter Coater, where a thin (10–30 nm) film of gold-palladium was evaporated onto the specimens. After sputter-coating, specimens were examined using an Amray 1830 scanning electron microscope and photographed on positive-negative film and paper.

Allonais inaequalis (Stephenson, 1911)
Figs. 1, 2

Material examined.—*Allonais paraguayensis*: Louisiana, 1963, and Florida, 1975, W. J. Harman collection from the National Museum of Natural History-Smithsonian Institution (USNM), Washington, D.C.; Nevis, Lesser Antilles, 1997, Illinois Natural History Survey (INHS) Annelida Collection, Champaign, identified by the authors; Sudan, Africa, 1975 and 1984, R. Grimm collection, Zoologisches Institut und Zoologisches Museum (ZMUH), Hamburg. *Allonais inaequalis*: Puerto Rico, 1994, INHS Annelida Collection, identified by the authors. *Allonais pectinata*: South Africa, 1968, Louisiana, 1974, 1975, Georgia,

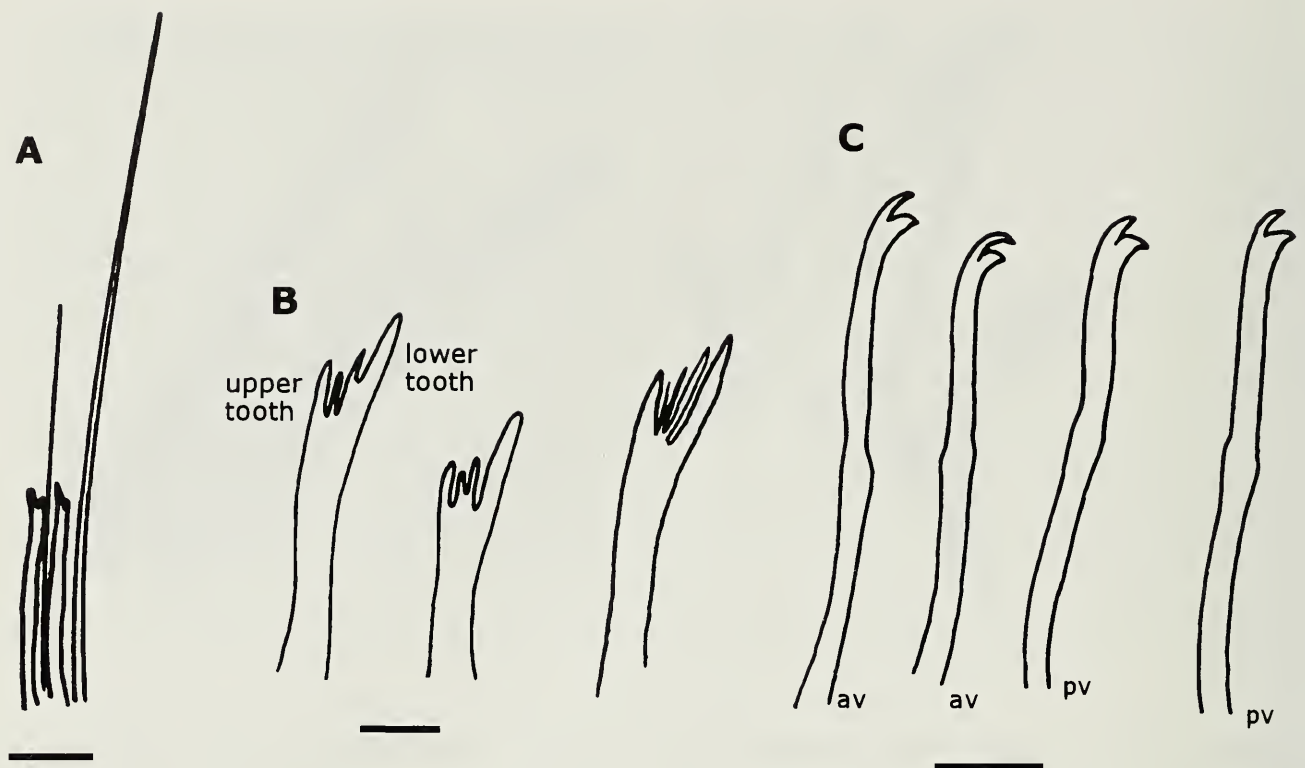


Fig. 2. Chaetae of *Allonais inaequalis*. A, dorsals; B, needles; C, ventrals. av, anterior ventral; pv, posterior ventral. Scale bars: A = 40 μm , B = 6.25 μm , C = 16 μm .

1967, and Florida, 1978, W. J. Harman collection from USNM. *Nais magnaseta* (holotype): Texas, 1973, USNM (45510).

Some specimens of *Allonais inaequalis* from New Jersey were deposited in the Annelida collection of Aquatic Resources Center, Thompsons Station, Tennessee, and the remainder were placed in the INHS Annelida Collection. The one specimen from Ohio was deposited in the collection at SoBran, Inc., Cincinnati, Ohio.

Redescription.—Body 3–9 mm long, consisting of 37–85 segments, no pigmentation. Prostomium rounded, without proboscis. No eyes. Dorsal chaetae begin in segment VI: 1–2 smooth hairs per bundle; if 2 hairs, then generally one longer (212–288 μm) and one shorter (75–160 μm); 1–2 needles per bundle, each usually with 2 intermediate teeth (but some with 1, 3, or 4), intermediate tooth next to upper is shortest of all teeth and intermediate tooth next to lower is second longest; needle length 64–96 μm , lower tooth (8–14 μm) almost twice as long as upper (5–8 μm). Ventral chaetae usually 5–7 per bundle (but as few

as 3 or as many as 9) in II–V, 55–75 μm long and 1–3 μm wide, nodulus slightly proximal, upper tooth slightly longer and thinner than the lower; from VI posteriorad usually 5–7 per bundle (but ranging from 3–9), 60–93 μm long and 1.5–3 μm wide (Puerto Rico specimens up to 5 μm wide), nodulus median to slightly distal, upper tooth thinner and subequal or slightly shorter than the lower; number of ventral chaetae decreases to 2 per bundle near the tail. No mature individuals were collected.

Discussion

It can be difficult to distinguish *Allonais inaequalis* from *A. paraguayensis* based on external morphological characters. Although Sperber (1948) stated that the sexual organs of these two species of *Allonais* are similar, there appear to be several characters that can be used to identify them as being different, as shown in Tables 1 and 2. The most obvious character is the number of intermediate teeth found in the needle chaetae. While the needle chaetae of *A. inae-*

Table 1.—Descriptions of the North American species of *Allonais* Sperber (1948) and Brinkhurst & Jamieson (1971) and of *Nais magnaseta* by Harman (1973). All measurements in μm unless otherwise indicated.

Character	<i>Allonais paraguayensis</i>	<i>Allonais inaequalis</i>	<i>Allonais pectinata</i>	<i>Nais magnaseta</i>
Anterior ventral chaetae:				
no./bundle	2–8 ^a ; 2–6 ^b	4–8 ^a ; 4–6 ^b	3–5	3–4
length	55–123	60–105	56–65	54
width		1.5	2	<2
teeth (upper vs. lower)	longer	longer	longer	slightly longer
Posterior ventral chaetae:				
no./bundle	2–8 ^a ; 2–6 ^b	4–8 ^a ; 4–6 ^b	2–7	2–5
length	55–123	60–105	51–58	57–67
width		1.5	2	3
teeth (upper vs. lower)	equal	equal	equal	thinner; slightly shorter
Dorsal chaetae:				
no. of hairs/bundle	1–2	1–2	1–2	1–2
length of hairs	200–500	100–332	70–220	114–180
no. of needles/bundle	1–2	1–2	1–2	1–2
length of needles	60–192	67–112	42–68	50–60
length, outer needle teeth			3.5	5–6
no. of intermediate teeth	1–2	1–4	1–5	1–3
Total length of worm, mm	4–60	8–18	1.5–8	2
No. of segments	15–200	40–95	15–65	31+
No. of penial chaetae	3–11	4–6	3–5	immature
No. of specimens				1

^a Brinkhurst & Jamieson (1971); ^b Sperber (1948).

qualis generally have two intermediate teeth (range 1–4; Figs. 1A, B, 2B), none of the specimens of *A. paraguayensis* examined had needle chaetae with intermediate teeth (Fig. 3). Sperber (1948) stated, however, that the upper tooth of *A. paraguayensis* can be bifid in some specimens. The posterior ventral chaetae generally are shorter and thinner in *A. inaequalis* (Fig. 2C) compared with those of *A. paraguayensis*; the upper teeth are always distinctly shorter than the lower teeth in *A. paraguayensis*, whereas in *A. inaequalis* the upper and lower teeth are generally subequal. The hair and needle chaetae are almost always shorter in *A. inaequalis* compared with *A. paraguayensis*. The needle chaetae of *A. pectinata* are distinct from those of its congeners, having both the upper and lower teeth subequal in length, and the intermediates recessed between the two.

Although most of the individuals examined from the USNM were in very poor condition, all of the specimens examined appear to be *A. inaequalis*, not *A. paraguayensis*, so it is very likely that the remaining specimens also are *A. inaequalis*. This means that *A. inaequalis* had already been found in North America (as early as 1963), but previously had been misidentified, and that *A. paraguayensis* has not yet been found in North America. Thus *A. inaequalis* can now be recorded from Florida, Louisiana, New Jersey, and Ohio, which suggests that it may be widespread but uncommon, at least in the eastern USA.

The specimens of *A. inaequalis* from North America fit the original description of Stephenson (1911) very well. All of his measurements fall within the ranges of ours, and he also noted the differences in length when two hair chaetae were present

Table 2.—Comparison of *Allonais paraguayensis* and *A. inaequalis* from specimens examined during this study. All measurements in μm unless otherwise indicated. If 2 hair chaetae present, then 1 is long and 1 is short. This agrees with the description of Stephenson (1911, 1920). First set of numbers for length of hairs reflects short hair; second set reflects long hair.

Character	<i>Allonais paraguayensis</i>					<i>Allonais inaequalis</i>	
	Sudan	Nevis	Florida, Louisiana ^a	New Jersey	Ohio	Puerto Rico	
Anterior ventral chaetae:							
no./bundle	2 (3)	3-4 (5)	3-5	3-6	4	4-5	
length	56-96	78-105	65-72	55-75	56-64	74-100	
width	2-3	1.5-3 (4)	1-1.5	2-2.5	2-2.5	2-3	
teeth (upper vs. lower)	thinner; subequal	thinner; subequal	thinner; slightly longer	thinner; slightly longer	thinner; slightly longer or subequal	thinner; slightly longer	
Posterior ventral chaetae:							
no./bundle	(3) 5-6	4-6 (7)	3-5 (7)	5-9	5-7	(3) 4-6	
length	80-118	88-105	64-75	60-70	64-72	75-93	
width	3-4	3-5 (6)	1.5-3	2-3	3	3-5	
teeth (upper vs. lower)	thinner; shorter	thinner; shorter	thinner; subequal or slightly shorter	thinner; subequal or slightly shorter	thinner; subequal or slightly shorter	thinner; subequal or slightly shorter	
Dorsal chaetae:							
no. of hairs/bundle	1-2	1-2	1-2	1-2	1-2	1 (2)	
length of hairs	110-198/225-353	150-200/205-325	95-125/212-250	88-160/213-275	75-112/238-262	75-80/224-288	
no. of needles/bundle	1-2	1 (2)	1-2	1-2	1-2	1-2	
length of needles	80-125	112-131	64-80	64-86	75-83	75-96	
no. of intermediate teeth	0	0	(1) 2-3	(1) 2 (3)	2 (3)	(1) 2-3 (4)	
Total length of worm, mm	ca. 3-4	ca. 5-8.5	ca. 2.6-4.5	ca. 3-9	posterior end missing	ca. 3-6	
No. of segments	35-90	60-118	42-61	37-85	posterior end missing	42-60	
No. of specimens	6	4	4	6	1	5	

^a Originally identified as *Allonais paraguayensis*.

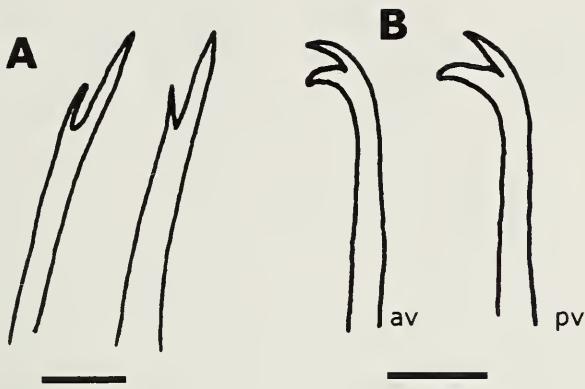


Fig. 3. Chaetae of *Allonais paraguayensis*. A, needles; B, ventrals. Abbreviations as in Fig. 2. Scale bars = 6.25 μm .

(“one of the hair-setae is much shorter than the other”; Fig. 2A). Sperber (1948), in erecting the genus *Allonais* and describing the species she ascribed to it, made no mention of the differences in length of the hair chaetae, so we do not know if it applies to all *Allonais* species or only to those that we examined. She stated, however, that she felt that *A. pectinata* belonged in a different, but new, genus.

Allonais pectinata, the third species in this genus reported to occur in North America, is reported to be widespread in the eastern USA (Illinois, Ohio, New York, Pennsylvania, and Georgia) and Ontario, Canada (Hiltunen & Klemm 1980, Brinkhurst 1986), yet neither of the present authors has ever seen a specimen in our many collections, and neither Klemm nor Brinkhurst (pers. comm.) was able to provide any specimens to corroborate its published distri-

bution. Because of the difficulty in distinguishing *A. pectinata* and *Nais magnaseta* Harman, 1973 from one another using the descriptions provided in the literature (Table 1), specimens of each species were obtained from the USNM for study.

Examination of the specimens from the USNM caused additional confusion. Two specimens from Lake Louisa at Clermont, Florida, previously identified as *A. pectinata*, were in fact *N. magnaseta* (see Table 3; Fig. 5), making this a new state record for the latter species, since it had previously only been reported from Bee County, Texas (Harman 1973). Another specimen from the USNM, collected from west of the Peach County line in Georgia and initially identified as *A. pectinata*, is actually *Nais variabilis* Piguët, 1906 with pectinate dorsal needle chaetae. The possible confusion of some specimens of *N. variabilis* (those with pectinate needle chaetae), and subsequent misidentification of them as *A. pectinata*, was noted previously by Brinkhurst (1986). The distinct difference in size and shape of the ventral chaetae in II–V compared with the rest of the ventrals in *N. variabilis* should easily separate those with pectinate needles from specimens of *A. pectinata*. Five specimens from Louisiana and three specimens from South Africa were examined and appear to be *A. pectinata*, although there are some differences between the two sets (see Fig. 4).

It is now clear that *A. pectinata* and *N.*

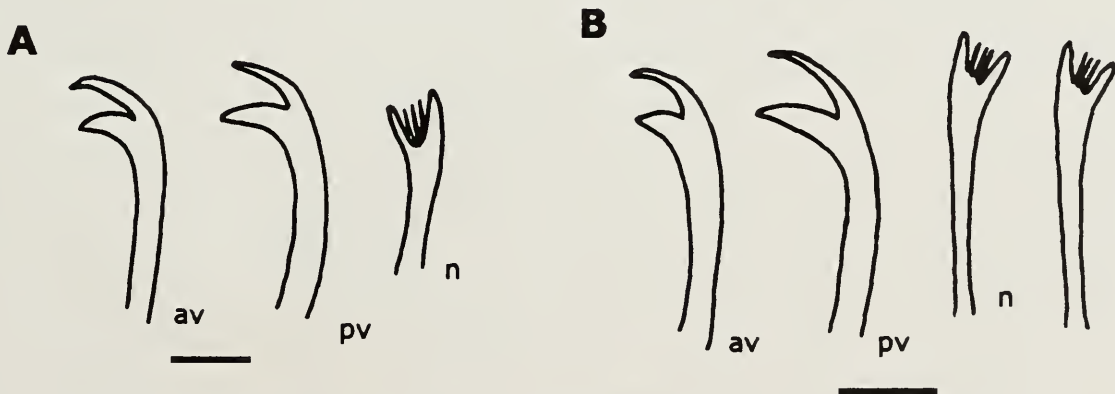


Fig. 4. Chaetae of *Allonais pectinata*. A, Louisiana specimen; B, South African specimen. av, pv as in Fig. 2; n, needle. Scale bars = 6.25 μm .

Table 3.—Comparison of *Allonais pectinata* and *Nais magnaseta* specimens examined during this study. All measurements in μm unless otherwise indicated.

Character	<i>Allonais pectinata</i>			<i>Nais magnaseta</i>	
	Louisiana	South Africa	Texas	Florida ^a	
Anterior ventral chaetae:					
no./bundle	(3) 4-5	2-4	3-4	3-4	
length	35-62	48-64	48-56	45-48	
width	1-1.25	1-1.5	1.25-1.5	1.25	
teeth (upper vs. lower)	slightly thinner; slightly longer	slightly thinner; equal or slightly longer	slightly thinner; equal or slightly longer	thinner; equal	
Posterior ventral chaetae:					
no./bundle	4-6	3-5	(2) 3-5	3-5	
length	48-64	54-83	53-64	48-56	
width	1.5-1.8	1.6-3	1.9-2.1	1.5-1.7	
teeth (upper vs. lower)	thinner; equal or slightly shorter	thinner; equal or slightly shorter	thinner; slightly shorter	thinner; shorter	
Dorsal chaetae:					
no. of hairs	1 (2)	1 (2) ^b	1	1	
length of hairs	93-112/128-176	112/168-256	128-160 ^c	192-216	
no. of needles/bundle	1-2	1-2	1	1-2	
length of needles	35-53	45-70	48-56	45-53	
no. of intermediate teeth	(2) 3	(2) 3	2-3 (?) ^c	2-4	
length, outer needle teeth	3-4.3	3-4.5	5-6.3	4-6.4	
No. of penial chaetae	4	immature	immature	immature	
Total length of worm, mm	1.4-2.1	1.7-2.3	1.5	1.5	
No. of segments	21+ to 36+	23+ to 37+	31+	36+	
No. of specimens	5	3	1	1 ^d	

^a Originally identified as *Allonais pectinata*; ^b if 2 hair chaetae present, then 1 is long and 1 is short; first set of numbers for length of hairs reflects short hair, second set reflects long hair; ^c very difficult to determine the actual number or measure length; ^d there were 2 specimens in this collection, but the second was partially obscured by the ringing material and therefore a definite identification could not be made.

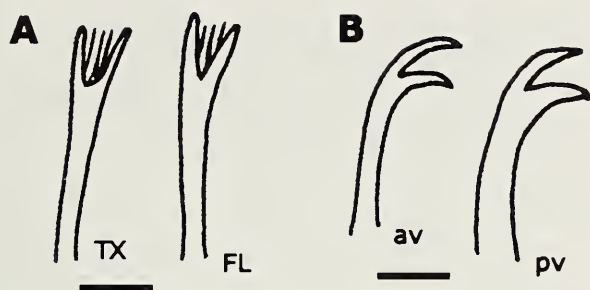


Fig. 5. Chaetae of *Nais magnaseta*. A, needles from Texas (TX) and Florida (FL) specimens; B, anterior ventral (av) and posterior ventral (pv), from Texas specimen. Scale bars = 6.25 μ m.

magnaseta are recognized as two distinct species (Table 3; Figs. 4, 5). The main differences between the two species are the length of the needle chaetae teeth, with those of *N. magnaseta* almost twice as long as those of *A. pectinata*, and the distinct difference in the length and width of the anterior ventral chaetae compared to the posterior ventral chaetae in *N. magnaseta*, while there is only a slight difference between the anterior and posterior ventral chaetae in *A. pectinata*.

Apparently most of the confusion associated with the identification of *A. inaequalis* and *A. paraguayensis* results from the original misidentification of the specimens from Louisiana and Florida. Although identified as *A. paraguayensis*, these obviously are *A. inaequalis* (Table 2). Brinkhurst (1986), followed by Kathman & Brinkhurst (1998), hinted at this possibility when they stated that the needle chaetae of *A. paraguayensis* from Louisiana seemed to resemble the needle chaetae of *A. inaequalis*. Hiltunen & Klemm (1980), assuming the identifications of *A. paraguayensis* to be correct, illustrated the needle chaetae of *A. inaequalis* but listed the species as *A. paraguayensis*. Adding to the confusion, the drawings of the needle chaetae of *A. inaequalis* by Sperber (1948) do not agree with the original drawing of Stephenson (1911) but resemble those of *A. pectinata*. Sperber referred to this as a form of *A. inaequalis*, but it is unclear if it really belongs with *A. inaequalis*. Kathman & Brinkhurst

(1998) added Florida, Illinois, and Colorado to the distribution list for *A. paraguayensis*. Examination of the Florida worms indicated that they were *A. inaequalis*, while those from Illinois and Colorado do not belong in *Allonais*. Brinkhurst (1986) and Brinkhurst & Marchese (1989) suggested that *A. inaequalis* had been synonymized with *A. paraguayensis* and *A. pectinata* but gave no references. In contradiction, Harman et al. (1988) stated that *A. inaequalis* clearly is distinct from *A. paraguayensis*. We do not know of any publications that have discussed possible synonymies of these two species, and Brinkhurst (pers. comm.) was unable to provide information about these comments.

Summary

Allonais inaequalis is herein reported for the first time in North America, although we believe that this species was found as early as 1963 but erroneously identified as *A. paraguayensis*. Its current distribution includes New Jersey, Ohio, Florida, and Louisiana. This means that *A. paraguayensis* has not yet been reported from North America. Although previously reported as fairly widespread, *A. pectinata* could only be confirmed as found in Louisiana. *Nais magnaseta*, known previously only from Bee County, Texas, has been identified from Florida.

Based on external morphological characters, it appears that the three *Allonais* species previously reported from North America can be easily differentiated, although *A. inaequalis* and *A. paraguayensis* seem to be more closely related to one another than to *A. pectinata*, as suggested by Sperber (1948). We know of no current phylogenetic analysis using either morphological characters or gene sequencing for this genus that would provide a more definitive approach to recognizing the species.

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